UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDE AND
TOXIC SUBSTANCES

This Report has been revised to reflect a change in the endpoint selection for occupational risk assessment through the inhalation route of exposure.

HED DOC.NO. 014595

DATE: June 18, 2001

MEMORANDUM

SUBJECT: ***Lindane*** **(PC Code: 009001)**- A Second Report of the Hazard Identification Assessment Review Committee.

FROM: Suhair Shallal, Toxicologist.
Reregistration Branch 4
Health Effects Division (7509C)

THROUGH: Elizabeth Doyle, Co-Chairman
and
Jess Rowland, Co-Chairman
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Mark T. Howard
Special Review and Registration Division

On May 22, 2001, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) met to reconsider the endpoint for occupation risk assessment for the inhalation route of exposure. Previously the endpoint was based on kidney lesions and increased kidney weights resulting from the accumulation of alpha 2µ globulin. These effects have been deemed not relevant for human risk assessment. The Committee's decision is presented in this report along with the previously conclusions of the June 13, 2000 HIARC meeting. In that meeting the Reference Dose (RfD) and the toxicological endpoints for acute and chronic dietary, as well as, occupational exposure risk assessments were selected. HIARC re-assessed the Reference Dose (RfD) established in 1994, as well as the toxicological endpoints selected for acute dietary and occupational/residential exposure risk assessments. The HIARC also

addressed the potential enhanced sensitivity of infants and children from exposure to lindane as required by the Food Quality Protection Act (FQPA) of 1996.

Committee Members in Attendance

Members present were: Elizabeth Doyle
David Nixon
Jess Rowland
Elizabeth Mendez
William Burnam
Pamela Hurley
Yung Yang
Brenda Tarplee
Jonathan Chen
Paula Deschamp

Member(s) in absentia: Ayaad Assaad

Data was presented by Suhair Shallal of the Reregistration Branch 4.

Also in attendance were: Susan Henley, Whang Phang, Joseph Nevola

Data Presentation:
and
Report Presentation

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Suhair Shallal,
Toxicologist

Report Concurrence:

_____
Brenda Tarplee
Executive Secretary

cc: RD
Casewell file

I. INTRODUCTION

On May 22, 2001, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) met to reconsider the endpoint for occupation risk assessment for the inhalation route of exposure. Previously the endpoint was based on kidney lesions and increased kidney weights resulting from the accumulation of alpha 2µ globulin. These effects have been deemed not relevant for human risk assessment. The Committee's decision is presented in this report along with the previously conclusions of the June 13, 2000 HIARC meeting. In that meeting the Reference Dose (RfD) and the toxicological endpoints for acute and chronic dietary, as well as, occupational exposure risk assessments were selected. HIARC re-assessed the Reference Dose (RfD) established in 1994, as well as the toxicological endpoints selected for acute dietary and occupational/residential exposure risk assessments. The HIARC also addressed the potential enhanced sensitivity of infants and children from exposure to lindane as required by the Food Quality Protection Act (FQPA) of 1996.

II. HAZARD IDENTIFICATION

A. Acute Reference Dose (RfD)* General population

Selected Study: Acute Neurotoxicity Study Guideline #: OPPTS 870.6200 [§81-8]

MRID No.: 44769201

Executive Summary: In an acute oral neurotoxicity study, groups of 10 Crl:CD®BR rats/sex/dose were administered single dose of Lindane (Batch No. HLS96/1, Purity 99.78%) by gavage at concentrations of 0 (control), 6, 20, or 60 mg/kg. Functional observational battery (FOB) and motor activity (MA) testing were performed prior to administration and within 3 hours (time of peak effect) of dosing (day 0), and on days 7 and 14 post-dose. Body weights were recorded pre-test, weekly during the study period and on FOB assessment days. Clinical signs were recorded at least once daily. At study termination all animals were sacrificed and fixed by whole body perfusion, designated tissues of the nervous system were processed for microscopic neuropathological evaluation.

All animals survived to scheduled termination. One male in the 60 mg/kg group was observed to convulse on the day of treatment within 2.75 hours after dosing. Clinical signs were also observed in females treated at 60 mg/kg within 24 hours of dosing and included: staining of the fur, stained urogenital region, hunched posture, and piloerection. These effects in females persisted for four

days. Significant treatment-related decreases in body weight gains were observed for males in the 60 mg/kg group compared to the control group for the first week of the study. Females administered this concentration also had slightly lower body weight gains throughout the study. Food consumption for males and females administered 60 mg/kg was significantly decreased compared to controls for Week 1 of the study. Food conversion ratios in the treated groups were not changed compared to control groups.

At the first FOB assessment on Day 0 (3 hours after dosing) males and females in the 60 mg/kg group exhibited piloerection (1 %, 2 &), decreased rectal temperature (1 %, 1 &), increased hindlimb foot splay and hunched posture (4 %, 7 &). Among males dosed at 60 mg/kg, increased respiration (3) and tremor/twitching (1) were observed. Females administered 60 mg/kg were observed to have increased incidences of walking on tip toes (10), licking behavior (3), decreased foot splay (3) and an absence of grooming (8) behavior. Females in the 20 mg/kg also had decreased grooming (3) behavior and increased forelimb grip strength (2). Motor activity was significantly decreased for males and females treated with 60 mg/kg as well as among females treated with 20 mg/kg three hours post-treatment. The 6 mg/kg group remained comparable to controls in FOB assessment parameters and MA.
No neuropathological changes were observed during the histological examinations of the peripheral or central nervous systems of these animals at any exposure concentration.

The NOAEL for systemic toxicity is 20 mg/kg for males and 6 mg/kg for females. Based on the substance-related effects on body weight, body weight gain, food consumption, and clinical signs of toxicity the LOAEL for systemic toxicity in males is 60 mg/kg. The LOAEL for females is 20 mg/kg based on a lower incidence of grooming behavior and decreased locomotor activity immediately after dosing, in addition to the parameters mentioned above.

The NOAEL for neurotoxic effects is 6 mg/kg for females and the LOAEL is 20 mg/kg based on increased forelimb grip strength and decreased grooming behavior and motor activity (MA). The NOAEL for neurotoxicity in males is 20 mg/kg and the LOAEL for males is 60 mg/kg based on tremors, convulsions, decreased MA, and increased forelimb grip strength.

This study is classified **Acceptable/guideline** and satisfies the Subdivision F guideline requirement for an acute oral neurotoxicity study (§81-8) in rats.

Dose and Endpoint for Establishing an Acute RfD:
The NOAEL is 6 mg/kg based on increased forelimb grip strength and decreased grooming behavior and motor activity in female rats

Uncertainty Factor(s): 100 ; 10X intraspecies variations and 10X interspecies etrapolation

Comments about Study/Endpoint/Uncertainty Factor(s):

Acute RfD = NOAEL (mg/kg) / UF = 6 / 100 = 0.06 mg/kg

Acute Reference Dose (RfD)* Females (13-50 years)

An appropriate endpoint attributable to a single dose could not be ascertained from either the developmental toxicity study in rats and in rabbits or in the developmental neurotoxicity study. Although, there was evidence of increased susceptibility in the DNT, the offspring effects were not attributable to a single dose. A separate endpoint for this subpopulation was therefore not identified.

2.2 Chronic Reference Dose (RfD)

Selected Study: Combined chronic toxicity/oncogenicity feeding – Rat

Guideline #: OPPTS 870.4300 [§83-5]

MRID No.: 41094101, 41853701 and 42891201

Executive Summary: Results from interim sacrifice of 15 rats/sex/group at 30 days and 26 weeks, as well as, 15 rats/sex/group at 52 weeks and final results of an ongoing chronic/oncogenicity study are presented in this report (MRID 41094101, 41853701 and 42891201).

In this chronic toxicity/oncogenicity study, Lindane (99.75% a.i., Lot no. DA433) was administered in the diet to groups of 115 male and 115 female Wistar rats at concentrations of 0, 1, 10, 100, or 400 ppm for 2 years. Corresponding delivered doses were 0, 0.05, 0.47, 4.81, and 19.66 mg/kg/day, respectively, for males and 0, 0.06, 0.59, 6.00, and 24.34 mg/kg/day, respectively, for females.

No clinical signs of toxicity were observed during the first 26 weeks; however by 52 weeks convulsions in 11 high-dose females were observed. No other clinical signs were observed. Survival at the end of the study was 36, 36, 31, 20, and 16% for males and 49, 38, 44, 35, and 18% for females in the 0, 1, 10, 100, and 400 ppm groups, respectively. Survival of high-dose males was similar to the controls through week 93. For females, however, survival was significantly decreased in the high-dose group with 50% survival reached at week 89 compared to week 104 for the control group.

Body weights were slightly less than the controls for the high-dose males (-6%) and females (-8%) during weeks 1-5 of the study, but gradually increased to within 2% of the control level by week 26 for males and week 9-10 for females. Because final body weights of the 100 ppm males were similar to the controls, the initial reduction in weight gain was not considered biologically significant. Final body weights of the high-dose males were significantly (-14%; $p \leq 0.05$) less than the controls. Body weights and body weight gains for the treated females were similar to the controls throughout the study. Food consumption by the high-dose groups was decreased 15% in males and 19% in females during the first week of the study, however, total food consumption for the entire study was similar to the control levels.

Platelet counts were significantly ($p \leq 0.05$ or 0.01) increased (20% or less) in the 100- and 400-ppm males at week 12 and in 100- and 400-ppm males and females at week 24, but not at later time points. High-dose males and females had significant ($p \leq 0.05$ or 0.01) decreases in red blood cell parameters at week 104 as compared with the controls: hemoglobin was -15.6% and -17.6%, respectively, erythrocyte counts were -14.1% and -21%, respectively, and PCV was -15.9% and -18.2%, respectively.

Significant ($p \leq 0.05$ or 0.01) changes in clinical chemistry parameters were observed in high-dose males and females during the first year of the study. Inorganic phosphorous was increased by 7.3-38.5% and calcium was increased by 3.4-10% in males and females; cholesterol was increased by 45-110% and urea was increased by 20-54% in females; and the albumin/globulin ratio was decreased by 8.3-18.2% in females. All parameters were similar to the control levels by week 104.

High-dose males and females had increased absolute and relative liver weights at all interim sacrifices, although statistical significance was not always reached. At study termination, absolute and relative liver weights were significantly ($p \leq 0.01$) increased by 21.2% and 38.5%, respectively, in high-dose males and by 31.6% and 33.5%, respectively, in high-dose females. At 100 ppm, absolute liver weights were increased by 8.6-11.2% (n.s.) and relative liver weights were increased by 14.4-17.6% ($p \leq 0.05$ or 0.01) for both sexes at week 104. Significant ($p \leq 0.05$ or 0.01) increases in absolute and relative spleen weights at week 52 and in relative spleen weights at week 104 were also noted, but the sex was not identified.

The incidence rate of periacinar hepatocytic hypertrophy was significantly increased in the 100- and 400-ppm groups with 25/50 males and 19/50 females affected at 100 ppm and 40/50 males and 43/50 females affected at 400 ppm. No treatment-related histopathological lesions were observed in the spleen or bone marrow.

Kidney lesions in males indicative of alpha 2μ globulin accumulation were observed in animals treated with 10 ppm.

Therefore, the systemic toxicity LOAEL for male and female rats is 100 ppm (4.81 and 6.0 mg/kg/day, respectively) based on periacinar hepatocyte hypertrophy, increased liver

and spleen weights, and decreased platelets. The systemic toxicity NOAEL is 10 ppm (0.47 and 0.59 mg/kg/day for males and females, respectively).

Eight additional males were identified as having adrenal pheochromocytomas. The revised percentages of animals with adrenal tumors in the 0, 1, 10, 100, and 400 ppm groups are 14, 16, 16, 6, and 24% for benign tumors, respectively, and 0, 0, 6, 8, and 2% for malignant tumors, respectively. Statistical significance was not reached by relevant tests. For comparison, historical control data from Charles River and publications in the open literature were submitted. The 10 and 100 ppm groups had malignant tumor incidence rates greater than the historical control rate (0-2%). The high-dose group also had a slight excess of benign and combined tumor rates as compared with the historical control rates (8-22% benign, combined could not be calculated), but this same net tumor incidence was the same as the control group of a published study. In the current study, pheochromocytomas were not considered the cause of death for any animal with the exception of a single animal in the 100 ppm group.

Therefore, no evidence of dose-related and statistically significant increase in adrenal tumors was observed in this study. The study was conducted at adequate dose levels.

Dose and Endpoint for Establishing a Chronic RfD: The NOAEL is 0.47 mg/kg/day **based on periacinar hepatocyte hypertrophy, increased liver and spleen weights, and decreased platelets in male rats**

Uncertainty Factor(s): 100; 10X intraspecies variations and 10X interspecies extrapolation

Comments about Study/Endpoint/Uncertainty Factor(s):
The HIARC concurred with the TES committee's decision (1994, DOC 013460) that the toxicological endpoint of concern was the periacinar hepatocyte hypertrophy and not kidney lesions associated with alpha 2μ globulin which is thought to be inappropriate for human risk assessment.

Chronic RfD = $\frac{\text{NOAEL (mg/kg/day)}}{\text{UF}} = \frac{0.47}{100}$ = **0.0047 mg/kg/day**

2.3 Occupational/Residential Exposure

2.3.1 Dermal Absorption

Selected Study: Dermal absorption study Guideline #: OPPTS 870.7600 [§85-3]

MRID No.: 40056107, 40056108

Executive Summary: In a dermal absorption study, 24 male Crl:CD®(SD)BR rats per

group received dermal applications of Lindane 20% emulsifiable concentrate ([^{14}C]-Lindane and unlabeled Lindane) at doses of 0.1, 1.0, or 10 mg/rat. Four animals/group were bled and sacrificed at intervals of 0.5, 1, 2, 4, 10, or 24 hours after application of the test article.

Quantities absorbed increased with dose and duration of exposure while percent absorbed increased with time and decreased with dose. Percents of the low-, mid-, and high-doses absorbed were 0.6, 0.96, and 0.66% after 0.5 hours; 18.07, 8.31, and 2.81% after 10 hours; and then, increased to 27.72, 20.86, and 5.05% after 24 hours. The total amount of test article absorbed after 24 hours, as calculated from urine, feces, and carcass, was 0.028, 0.21, and 0.51 mg for the low-, mid-, and high-dose groups, respectively. The process appears to be approaching saturation at the high dose. Recovered radioactivity (absorbed, skin, skin rinse, filter paper and spreader) was 74.19, 70.19 and 58.35% of the applied dose after 24 hours of exposure in the low-, mid-, and high-dose, respectively.

This study is considered **Acceptable/guideline** and satisfies the requirements for a dermal absorption study in rats [85-2].

Percent Dermal Absorption by Rats Based on Exposure and Duration

Applied dose/rat (mg/kg)	Exposure Duration		
	4 hr	10 hr	24 hours
0.1 mg (.25 mg/kg)	10.1	18.1	27.7%
1.0 mg (2.5 mg/kg)	5.3	8.3	20.9%
10.0 mg (25 mg/kg)	2.0	2.8	5.0%

Executive Summary: In a dermal absorption study, 24 male Hra: (NZW) SPF rabbits per group received dermal applications of Lindane 20% emulsifiable concentrate ([^{14}C]-Lindane and unlabeled Lindane) at doses of 0.5, 5.0, or 50 mg/rabbit. Four animals/group were bled and sacrificed at intervals of 0.5, 1, 2, 4, 10, or 24 hours after application of the test article.

Quantities absorbed increased with dose and duration of exposure while percent absorbed increased with time and decreased with dose. Percentages of the low-, mid-, and high-doses absorbed were 5.97, 6.68, and 1.99% after 0.5 hours; 51.68, 23.76 and 10.96% after 10 hours; and then increased to 55.68, 39.99, and 16.56% after 24 hours. The total amount of test article absorbed after 24 hours, as calculated from urine, feces, and carcass, was 0.28, 2.00, and 8.46 mg for the low-, mid-, and high-dose groups, respectively. The original DER states that No evidence of saturation of

the absorption process was observed; however upon further examination it appears that there is evidence of saturation at the highest dose (50 mg/rabbit) tested. Recovered radioactivity (absorbed, skin, skin rinse, filter paper and spreader) was 82.01, 78.27 and 66.34% of the applied dose after 24 hours of exposure in the low-, mid-, and high-dose, respectively.

This study is considered **Acceptable/guideline** and satisfies the requirements for a dermal absorption study in rabbits [85-2].

At 10 hours, 18% of the applied material is absorbed. IPCS (1991) sites even higher percentages at 24 hours, ranging from 28% for rat and 17 to 56% for rabbit.

Percentage (%) Dermal Absorption:

The absorption has been determined to be 10%.

Comments about Dermal Absorption:

The HIARC concurred with the TES committee decision (HED Doc. # 013460) that the dermal absorption factor is 10% based on a published report by Feldman and Maibach (Toxicology and Applied Pharmacology 28, 126-132, 1974).

The Maibach study tested 12 pesticides and herbicides, including Lindane, on human subjects (6 per chemical) to quantitate their dermal penetration. C^{14}-labeled chemicals were applied topically ($4\mu g/cm^2$) to the forearm or via the intravenous route ($1\mu Ci$). Excretion of the chemicals was then monitored by collecting and analyzing urine samples during the 5 day testing period. All results were calculated as percent of the injected or applied dose. Data obtained after IV dosing was used to correct the skin penetration data for incomplete urinary recovery. Lindane was shown to have a penetration factor of 9.3% ± 3.7 (SD).

2.3.2 Short-term Dermal (1 - 7 days) Exposure

Selected Study: Developmental Neurotoxicity Study

Guideline #: OPPTS 870.6200 [§83-6]

MRID No.: 45073501

Executive Summary: In a developmental neurotoxicity study (MRID 45073501), lindane (Batch No. HLS 96/1; 99.78% a.i.) was administered to presumed pregnant Hsd Brl Han:Wist (Han Wistar) rats in the diet at concentrations of 0, 10, 50, or 120

ppm from gestation day (GD) 6 through lactation day 10. These concentrations resulted in F_0 maternal doses of 0.8-0.9, 4.2-4.6, and 8.0-10.5 mg/kg/day, respectively, during gestation and 1.2-1.7, 5.6-8.3, and 13.7-19.1 mg/kg/day, respectively, during lactation. The developmental neurotoxicity of lindane was evaluated in the F_1 offspring. F_1 animals (10/sex) were evaluated for FOB, motor activity, auditory startle response, and learning and memory as well as developmental landmarks such as vaginal perforation and balanopreputial separation, and brain weights and histopathology on days 11 and 65, including morphometrics.

Small differences in absolute maternal body weights (7-8%) were observed between the high dose and control groups during gestation and early lactation (through day 11). Body weight gains by the high-dose dams from GD 6 through GD 20 were 64-79% ($p \leq 0.01$) of the control level. Body weight changes during lactation were similar between the treated and control groups. During gestation, food consumption by the high-dose group was significantly ($p \leq 0.01$; 74-92% of controls) less than the control group for the intervals of GD 10-13, 14-17, and 18-19. Food consumption by the low- and mid-dose groups during gestation and by all treated groups during lactation was similar to the controls.

Absolute body weights of the treated male and female pups in mid and high dose groups during lactation were 12-18% and 16-20% less than controls, respectively on days 4-11 of lactation with recovery to less than 10% by day 21. Body weight gains ($p \leq 0.05$ or 0.01) on lactation days 1-4 and 1-11 were 76% and 84%, respectively, of the control levels for mid-dose males, 79% and 79%, respectively, for mid-dose females, 60% and 73%, respectively for high-dose males, and 63 and 75%, respectively, for high-dose females. Body weight gains by all treated groups were similar to the controls during lactation days 11-21. Except for mid and high dose females, postweaning, body weight gains were similar between the treated and control groups. Body weight differences for high dose dams were 10% less at the beginning of lactation and recovered to 6% less by the end of the study. The high-dose group had a greater number of stillborn pups as indicated by a live birth index of 77% compared with 99% for the control group. In addition, nine high-dose litters either died or were sacrificed moribund on lactation days 1-4. This resulted in a viability index for the high-dose group of 71% compared with 89% for the controls. Pup mortality in the mid and high-dose groups in litters surviving to weaning was greater before day 4 than in controls [3 pups in 2/20 controls; 18 pups in 8/22 litters, mid dose; 14 pups in 4/15 litters, high dose]. Survival was not affected at any time in the low dose group as compared with the control group. No dose- or treatment-related differences were observed between treated and control groups for duration of gestation, number of pups/litter on day 1, or per cent male offspring. At necropsy, no treatment-related gross abnormalities were observed in the dams or offspring. Absolute and relative liver and kidney weights of the offspring were not affected by treatment.

A few clinical signs were observed in high dose dams and pups; increased reactivity to

handling in dams on weeks 2 and 3 of dosing, and slower surface righting in pups on day 4. There were no effects on measures of physical or sexual development. There was an increase in motor activity at the mid and high dose during lactation in both sexes. Some decrease in habituation of motor activity in females on day 22 was also seen. While there was no effect on auditory startle reflex amplitudes, there was a clear reduction in auditory startle response habituation in both sexes at the high dose on day 28 and on day 60. Slight decreases in absolute, but not relative, brain weights in mid and high dose female pups were observed on postnatal day 11 (9-10%) but narrowed to 3-5% less by day 65. Brain lengths and widths were similar between the treated and control pups. Morphometric brain measurements did not show any significant differences in the sizes of the neocortex, hippocampus, corpus callosum, or cerebellum on days 11 or 65. There were no effects on histopathology of the nervous system.

The maternal toxicity LOAEL is 120 ppm (13.7 mg/kg/day) based on decreased body weight gains, decreased food consumption, and increased reactivity to handling. The maternal toxicity NOAEL is 50 ppm (5.6 mg/kg/day).

The developmental toxicity LOAEL is 50 ppm (5.6 mg/kg/day) based on reduced pup survival, decreased body weights and body weight gains during lactation, increased motor activity, and decreased motor activity habituation. The developmental toxicity NOAEL is 10 ppm (1.2 mg/kg/day).

This study is classified as **Unacceptable/Guideline** [870.6300 (§83-6)] since laboratory validation studies of the neurobehavioral tests were not included, but it may be upgraded and found acceptable if this information is obtained. The number of animals tested at the highest dose is only 6 compared to the required number of 10 animals per dose.

Dose and Endpoint for Risk Assessment: The NOAEL is 1.2 mg/kg based on reduced pup survival, decreased body weights and body weight gains during lactation, increased motor activity, and decreased motor activity habituation.

Comments about Study/Endpoint/Uncertainty Factor(s):
A 90-day dermal toxicity study in rabbits was available; the NOAEL was 10 mg/kg/day and the LOAEL was 60 mg/kg/day based on hepatic toxicity. The HIARC did not consider this study to be appropriate for risk assessment and instead selected an oral endpoint due to:

1) the concern for developmental effects as seen in pups in the developmental neurotoxicity study
2) developmental effects are not evaluated in the dermal toxicity study
3) the dermal toxicity study was conducted in the rabbit, while the increased

susceptibility was seen in rat pups via an oral route
4) this endpoint will be protective of dermally exposed workers

Since an oral endpoint was selected, a 10% dermal absorption factor should be used for route to route extrapolation. Although this study is classified as unacceptable/guideline, it is adequate for endpoint selection because the deficiencies are related to submission of additional data and not the quality of the study.

2.3.3 Intermediate-term Dermal (1-Week to Several Months)

Selected Study: Developmental Neurotoxicity Study in rats

Dose and Endpoint for Risk Assessment:
The NOAEL is 1.2 mg/kg based on reduced pup survival, decreased body weights and body weight gains during lactation, increased motor activity, and decreased motor activity habituation.

Comments about Study/Endpoint: See Short-term Dermal Section 2.3.2

2.3.4 Long-term Dermal (Several Months to Lifetime)

Selected Study: Chronic toxicity and Oncogenicity Study in rats

MRID No.: 41094101, 41853701 and 42891201

Dose and Endpoint: The NOAEL is 0.47 mg/kg/day **based on periacinar hepatocyte hypertrophy, increased liver and spleen weights, and decreased platelets in male rats**

Comments about Study/Endpoint: This dose and endpoint was used to derive the chronic RfD. Since an oral NOAEL was selected, a 10% dermal absorption factor should be used.

2.4 Inhalation Exposure (All Durations)

Short-term (1-7 days):

Selected Study: Subchronic Inhalation Toxicity Study

Guideline #: OPPTS 870.3465 [§82-4]

Accession No.: 255003

Executive Summary: In a subchronic inhalation toxicity study (Accession No. 255003), Lindane (99.9% a.i., Batch no. 79044/174) was administered by inhalation to groups of 12 male and 12 female Wistar rats at nominal concentrations of 0, 0.02, 0.10, 0.50, or 5.0 mg/m^3, 6 h/day for 90 days. Additional control and high concentration groups, 12 rats/sex, were treated for 90 days and allowed to recover for 6 weeks before sacrifice. Analytically measured atmospheric concentrations were 0, 0.02, 0.12, 0.60, and 4.54 mg/m^3, respectively. The arithmetic mean particle size of the aerosol was 1.11±0.39 ：m and the geometric mean was 1.03±1.45 ：m.

Lindane was detected in the brain, liver, fat, and serum of all exposed rats. The chemical accumulated in fat with levels reaching 127,120 ：g/g and 58,260 ：g/g in high-dose females and males, respectively. After the recovery period, traces of lindane were still detectable in the tissues.

All rats survived to scheduled sacrifice. "Slight" diarrhea and piloerection were observed in all males and females exposed to the highest concentration, beginning at 14 days after exposure and continuing for 20 days. No exposure-related effects were noted for body weight gain, food consumption, water consumption, or urinalysis parameters. Although hematology parameters did appear to be affected by treatment, no individual animal data were included and the statistics could not be verified. Clinical chemistry results, especially for Na^+, K^+, and Ca^{++}, were highly variable. Cytochrome p-450 in males and females exposed to 5 mg/m^3 was 338% and 174%, respectively, of the control values after 90 days, but similar to the control levels after the recovery period.

Bone marrow myelograms from animals exposed to 5 mg/m^3 showed significantly (p # 0.05) increased reticulocytes (+108%), stem cells (+31%), and myeloblasts (+33%) in males,and increased reticulocytes (+55) in females, and decreased (-45%) lymphocytes in females. However, these changes in bone marrow cannot be definitively attributed to treatment since bone marrow from the other exposed groups was not assayed.

Males exposed to 5 mg/m^3 had significantly (p # 0.05 or 0.01) increased absolute (+7.8% to +11.7%) and relative (+19.1% to 19.2%) kidney weights as compared with the controls. Absolute and relative kidney weights in the males exposed to 0.5 mg/m^3 were increased by 8-9.8% and 6.9-8.2%, respectively. Although not statistically significant, the increases in kidney weights for these groups were considered biologically significant. After the recovery phase, kidney weights from the exposed males were similar to the controls. In females exposed to 5 mg/m^3 absolute and relative kidney weights were increased (p # 0.05) by 9.2-9.9% and 7.9-8.2%, respectively, as compared with the controls.

In high-dose males, absolute liver weights were not affected, but relative liver weights were slightly (6.9%) higher than the controls. For females exposed to the highest dose, absolute and relative liver weights were 12.2% and 11.0% higher, respectively, than the controls. No differences in absolute and relative liver weights were noted between the exposed and control groups after the recovery period.

Kidney lesions in males exposed to 0, 0.02, 0.10, 0.50, or 5.0 mg/m^3, were observed in 17%, 0, 25%, 83% and 82%, respectively, of the animals. These lesions included cloudy swelling of the tubule epithelia, dilated renal tubules with protein containing contents, and proliferated tubules. After the recovery phase, only cloudy swelling of the tubule epithelia was observed in two control animals and one high-concentration animal. These effects are consistent with the accumulation of alpha 2µ globulin and is not relevant for human risk assessment.

Therefore, the systemic toxicity LOAEL is 5.0 mg/m^3 based on increased kidney weights of female rats and bone marrow effects. The systemic toxicity NOAEL is 0.5 mg/m^3.

This study is considered **Acceptable/guideline** and satisfies the requirement for a subchronic inhalation toxicity study in rats [82-4]. It should be noted that several translation errors were found and corrected by referring to the original text. Individual animal data were not available for statistical analysis of blood elements or clinical chemistry data.

Dose and Endpoint for Risk Assessment: **The NOAEL is 0.5 mg/m^3** (0.13 mg/kg) based on clinical signs (diarrhea and piloerection) seen at day 14 after exposure and continuing for 20 days.

Comments about Study/Endpoint:
The HIARC established a NOAEL of 0.5 mg/m^3 for this risk assessment based on clinical signs seen at the highest concentration tested (5 mg/m^3). This NOAEL is applicable and appropriate only for short-term exposure risk assessment because the effects were seen during this period of exposure. The Committee further noted that this dose would be protective against developmental effects.

Intermediate term (7 days to several months):

Selected Study: Subchronic Inhalation Toxicity Study

Accession No.: 255003

Dose and Endpoint for Risk Assessment: **The NOAEL is 0.5 mg/m^3** (0.13 mg/kg) based on increased kidney weights in females and bone marrow effects (increased reticulocytes, increased myelocytes, decreased lymphocytes) at 5 mg/m^3.

Comments about Study/Endpoint:
The NOAEL of 0.1 mg/m^3 based on kidney lesions and increased kidney weights in male rats at 0.5 mg/m^3 , selected previously on June 13th, 2001 HIARC meeting, has been changed. The change in endpoint selection was necessary because the kidney effects are due to the accumulation of alpha 2µ globulin, a low molecular weight protein in the male rat kidney, and this accumulation initiates a sequences of events that may lead to tumor formation. This phenomenon does not occur

in female rats. The Agency has determined that in this special situation, the male rat is not a good model for assessing human risk (USEPA, 1991).

The route and duration of exposure in this study is appropriate for this exposure scenario.

Long Term Inhalation:

Based on the use pattern (maximum of 60 days), no long-term inhalation exposure is expected. If there is a change in the use pattern and a long-term exposure becomes likely, then the inhalation NOAEL of 0.5 mg/m^3 (0.13 mg/kg) should be used for risk assessment.

Recommendation for Aggregate Exposure Risk Assessments

There are no registered residential uses at this present time; therefore, non-occupational aggregate exposure risk assessment will be limited to food and water.

For occupational risks, separate assessments should be conducted for dermal and inhalation exposures because the effects selected for assessment of dermal risk do not share a common toxicity with the effects selected for inhalation risk.

Margins of Exposures for Occupational/Residential Exposure Risk Assessments

An MOE of 100 is adequate for both dermal and inhalation occupational exposure at all time durations.

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

3.1 Combined Chronic Toxicity/Carcinogenicity Study in Rats

MRID No.: 41094101, 41853701 and 42891201

Executive Summary: See Chronic RfD section

Discussion of Tumor Data: No tumors were noted in this study. The findings included a significant increase in the incidence rate of periacinar hepatocytic hypertrophy in the 100- and 400-ppm groups with 25/50 males and 19/50 females affected at 100 ppm and 40/50 males and 43/50 females affected at 400 ppm. No treatment-related histopathological lesions were observed in the spleen or bone marrow. For further details, please refer to the chronic RfD section-2.2.

Adequacy of the Dose Levels Tested: The dose levels are adequate to assess the carcinogenic potential of Lindane.

3.2 Carcinogenicity Study in Mice NO ACCEPTABLE STUDY IS AVAILABLE

Comments and Discussion: A new mouse carcinogenicity study is expected in December 2000

3.3 Classification of Carcinogenic Potential

A new Cancer Assessment Review Committee (CARC) meeting will review the recently submitted mouse carcinogenicity study and establish a new classification for lindane, if applicable. According to the TES committee report (1994, Doc 013460), Lindane has not been classified by the HED Cancer Peer Review Committee. It was determined by the RfD/Peer Review Committee (8/25/93) that: "The mouse carcinogenicity data were considered insufficient because of major deficiencies associated with all studies available." Lindane however had been previously classified by the Cancer Assessment Group of the Office of Research and Development (memorandum dated 7/23/85 from R.E. McGaughy to Anne Barton) as a group B2/C carcinogen based on increased incidence of mouse liver tumors. The upper-bound slope of the dose-response was given in that memorandum as Q1* = 1.1 $(mg/kg/day)^{-1}$.

IV. MUTAGENICITY

Executive Summary: In a mammalian cell gene mutation assay (MRID 00144500) conducted in Chinese hamster V79 cells, lindane was tested in the absence of metabolic activation at dose levels of 2.5, 5, 10, 25, 50, 70, 100, and 150 : g/ml and in the presence of metabolic activation at dose levels of 5, 10, 25, 50, 100 250 and 500 : g/ml. The S9 fraction used for metabolic activation was obtained from Aroclor 1254-induced mouse liver. Tests with and without activation were conducted under aerobic and anaerobic conditions.

Under anaerobic conditions, lindane without S9 was cytotoxic to the V79 cells at dose levels above 10 : g/ml and with S9 at dose levels above 150 µg/ml. **No mutagenic activity of lindane was observed in V79 cells** under any combination of conditions up to cytotoxic doses. No statistical analysis was performed; solvent control values were somewhat variable; the positive control values were appropriate for the experiments under aerobic conditions, but the positive control employed for anaerobic conditions did not exhibit an increase in anaerobic mutation frequency compared to aerobic mutation frequency.

Moreover, there was no experimental verification that anaerobic conditions were either established before exposure to lindane or maintained throughout the exposure period. Since the

anaerobic positive control did not produce more mutations under anaerobic than under aerobic conditions, anaerobic metabolic pathways may not have been induced in the cells.

This study is classified as **Unacceptable/Guideline** and does not satisfy guideline requirements for a mammalian cell culture gene mutation assay in V79 cells (84-2) because of the deficiencies described above. This classification could not be upgraded without repeating the experiments.

Executive Summary: In a mammalian *in vivo* sister chromatid exchange (SCE) assay (MRID 00024504), 50 : g tablets of bromodeoxy-uridine were implanted into male and female CF-1 mice. Two hours after implantation, lindane was administered *ip* in arachis oil at dose levels of 1.3, 6.4 and 32.1 mg/kg. These doses were reported to be 1/75, 1/15 and 1/3 of the LD_{50}. The vehicle control group received arachis oil and the positive control group received 10 mg/kg of cyclophosphamide in saline. Colcemid was administered 22 hours later to arrest cells in mitosis, and after another 2 hours the animals were sacrificed. For each dose level and control group, 30 bone marrow cells from each of 5 animals of each sex were examined for SCEs.

No toxicity was reported in any treatment group. Slight but significant increases in SCEs over the vehicle controls were observed in female but not in male animals at all dose levels tested but were not dose-related (1.29 [*sic*], 1.82, and 2.12 SCE/cell). Vehicle control values for female animals were also found to be significantly lower than those for males (1.56 ± 0.089 SCE/cell compared to 1.86 ± 0.207 SCE/cell). When results for male and female animals were pooled, only the highest dose produced a significant increase in SCEs over the controls. Positive control values were appropriate.

The study authors concluded that no chromosome damage was observed in this test.

This study was classified as **Acceptable/Guideline** and satisfies the guideline requirements for a sister chromatid exchange study in mice (*in vivo* SCE) (84-2).

Executive Summary: In a mammalian dominant lethal assay (MRID 00062657), 10 male Sprague-Dawley rats of unspecified age per group were exposed to lindane administered by subcutaneous injection in corn oil at doses of 0, 1, 3, and 10 mg/kg five time per week for 10 weeks. Immediately following treatment, each male was housed with two virgin females. After one week, the females were replaced with two more virgin females. No positive control group was included in the study. Females were sacrificed 14 days after evidence of mating or, lacking evidence, 14 days after removal from males. Uteri were examined for live and dead implants and abnormalities. Males were also sacrificed and gross pathological analysis performed.

Very slight but not statistically significant weight loss was observed in the male animals at the two higher doses. No mortality or treatment-related clinical signs of toxicity were noted. No treatment related effect on pregnancy rate was observed, although pregnancy rates in all groups

were low during the first week. The incidence of dead implants was significantly increased at the lowest dose but not at the two higher doses in the first week of mating but this increase was not observed during the second week. **The authors conclude that lindane did not cause an increase in the incidence of dominant lethals in this study.**

This study is classified as **Unacceptable/Guideline** and does not satisfy the guideline requirements for a dominant lethal test in the rodent (84-2) because no positive control was done, the criteria for toxicity were inadequate, animal age was not given, and insufficient numbers of pregnant dams were produced for meaningful evaluation. Moreover, no rationale was provided for the dose selection, unusual route of administration or dosing regime. This classification could not be upgraded without repeating the study.

IPCS has also determined that Lindane does not appear to have mutagenic potential.

V. FQPA CONSIDERATIONS

5.1 Adequacy of the Data Base

-- Acute delayed neurotoxicity study in hen (if applicable)
-X- Acute and subchronic neurotoxicity studies (if applicable)
-X- Developmental toxicity studies in Rat & Rabbits
-X- Two-Generation Reproduction Study
-X- Developmental neurotoxicity study (if applicable)

THESE STUDIES ARE AVAILABLE AND THE DATA BASE IS ADEQUATE FOR FQPA EVALUATION OF FQPA.

5.2 Neurotoxicity Data

1- Acute Neurotoxicity -§81-7: See Acute RfD section

2- Subchronic Neurotoxicity- §82-5

MRID: 44781101

Executive Summary: In a subchronic oral neurotoxicity study (MRID 44781101), groups of

10 Crl:CD®BR rats/sex/group were administered Lindane (Batch No. HLS96/1, Purity 99.78%) in the diet for 13 weeks at concentrations of 0 (control), 20, 100, or 500 ppm. Due to severe toxic reactions to treatment at 500 ppm, the dose was reduced to 400 ppm on day 11 of treatment thereafter. These doses resulted in average daily intake values of 0, 1.4, 7.1, and 28.1 mg/kg/day for males and 0, 1.6, 7.9, and 30.2 mg/kg/day in females for 0, 20, 100, and 500/400 ppm, respectively. Functional observational battery (FOB) and motor activity (MA) tests were performed prior to administration and after 4, 8, and 13 weeks of treatment. Body weights were recorded pre-test, weekly during the study period and on FOB assessment days. Clinical signs were recorded at least once daily. At study termination all animals were sacrificed and fixed by whole body perfusion and designated tissues of the nervous system were processed for microscopic neuropathological evaluation.

Three females in the 500/400 group died prior to scheduled termination. These deaths were attributed to treatment with Lindane. One death was recorded on Day 11 of the study, one during week 10 and one during week 13. Clinical signs prior to death included weight loss, swollen muzzle with scabbing, hunched posture, piloerection, and staining of the anogenital region. Observations in surviving females treated at 500/400 ppm were hypersensitivity to touch, staining of the urogenital region, and scabbing of the toes.

Significant treatment-related decreases ($p<0.05$ or $p<0.01$) in body weight were observed among males and females treated with 500/400 ppm of 14% and 23%, respectively. Decreases in body weight gains (70% **%** and 180% **&**, $p<0.01$), food consumption (35% **%** and 50% **&**, $p<0.05$ or $p<0.01$, respectively), and food conversion ratios were observed for males and females in the 500 ppm groups compared to the control group for the first week of the study. Male rats tended to recover from these effects after the dose was lowered. Females, however, did not exhibit this same level of recovery as their food consumption remained slightly depressed throughout the remainder of the study.

Females in the 100 ppm group had significantly decreased body weight gains (40%, $p<0.05$) compared to the control group during the first week of the study and this effect continued, although not at a level of significance throughout the remainder of the study. Females in the 100 ppm group had significantly decreased food consumption (16%, $p<0.01$) for the first week of the study and this trend continued throughout the study. Liver weights were also found to be increased at 500/400 ppm for both sexes; no additional information was given.

During the FOB assessment (table A is attached at the end of this document), males and females treated at the highest dose (500/400 ppm) were perceived as difficult to handle. They also were observed to have piloerection and hunched posture. Females in the highest dose group had missing claws (3), tended to urinate more often than controls, had a higher incidence of grooming behavior, rearing, motor activity, and one female was observed to convulse. Females across the dose groups were observed walking on tiptoes (5-7) and these incidences

were significantly increased compared to the control (1) for the highest dose group. Females (5) in the 100 ppm group also had increased incidences of grooming behavior at the Week 4 evaluation and one animal in this group was extremely difficult to handle.

The assessments of forelimb and hindlimb grip strength as well as hindlimb splay revealed no differences for any of the treated groups compared to the control groups. Colburn motor activity was also similar among treated groups compared to the control groups.

No neuropathological endpoints attributable to Lindane administration were observed during the histological examinations of the peripheral or central nervous systems of these animals at any exposure concentration.

The NOAEL for systemic toxicity is 100 ppm for males (7.1 mg/kg) and 20 ppm for females (1.6 mg/kg). Based on the substance-related effects on body weight, body weight gain, food consumption, and clinical signs of toxicity the LOAEL levels for systemic toxicity in males is 500/400 ppm (28.1 mg/kg)and 100 ppm for females (7.9 mg/kg).

The NOAEL for neurotoxic effects is 100 ppm for males (7.1 mg/kg) and females (7.9 mg/kg). The neurotoxicity LOAEL is 500/400 ppm based on hypersensitivity to touch and hunched posture.

This study is classified **Acceptable/guideline** and satisfies the Subdivision F guideline requirement for an acute oral neurotoxicity study (§81-8) in rats.

5.3 Developmental Toxicity

Executive Summary: In a developmental toxicity study (MRID 42808001), 20 presumed pregnant CFY (derived from Charles River CD) rats per group were administered technical Lindane (purity not given; Batch No. 6801/403) by gavage in 0.5% carboxymethyl-cellulose at doses of 0, 5, 10, and 20 mg/kg/day on gestation days (GD) 6-15, inclusive. On GD 20, dams were sacrificed by CO_2, subjected to gross necropsy, and all fetuses examined externally. Approximately one-third of each litter was processed for visceral examination and the remaining two-thirds was processed for skeletal examination.

Deaths of two high-dose dams were attributed by the authors to treatment although the cause of death was not reported. No treatment-related clinical signs of toxicity were observed in any animal. Body weight gains and food consumption by the mid- and high-dose groups were decreased during the treatment interval as compared with the controls. Body weight gains by the mid- and high-dose dams were 70% and 46%, respectively, of the control values during GD 6-14. Food consumption by the mid- and high-dose groups was 72% of the control level

during GD 7-10 and 92% and 65%, respectively, during GD 11-14. It should be noted that data were not available for the entire dosing interval and that statistical analyses were not provided for these data.

Maternal necropsy was unremarkable. Organ weights were similar between the treated and control groups.

Therefore, the maternal toxicity LOAEL is 10 mg/kg/day based on reduced body weight gain and food consumption. The maternal toxicity NOAEL is 5 mg/kg/day.

No significant differences were observed between the control group and the treated groups for number of corpora lutea, number of implantation sites, live fetuses/dam, pre- and post-implantation losses, fetal body weights, or fetal sex ratios. No treatment-related effects were found at external or visceral examination of the fetuses.

The percentage of litters in the control, low-, mid-, and high-dose groups containing fetuses with extra (14th) ribs was 12.7, 21.0, 31.7, and 40.6% (p 0.05), respectively. The total incidences of litters containing fetuses with skeletal variants were 43.4, 52.7, 59.5, and 68.0% (p 0.01), respectively. Although the response rates in the high-dose group for extra ribs and total variants are within the upper limit of historical control data, they were considered treatment-related due to the dose-related manner of increase.

Therefore, the developmental toxicity LOAEL is 20 mg/kg/day based on increases in extra ribs and total skeletal variants; a trend for increases in these endpoints at the lower doses is recognized. The developmental toxicity NOAEL is 10 mg/kg/day.

This study is classified as **Acceptable/nonguideline** and does satisfy the requirements for a developmental toxicity study (83-3a) in rats. Several deficiencies were noted in the conduct of this study: percent purity of the test article was not given, less than 20 litters/group were available, dosing solutions were not analyzed for concentration, stability, or homogeneity, and much of the individual animal data were not included. This study was conducted prior to implementation of current guidelines.

Executive Summary: In a developmental toxicity study (MRID 00062658), groups of presumed pregnant Sprague-Dawley rats were administered Lindane (purity not given; Lot No. 36346) by subcutaneous injection in corn oil (1 ml/kg) at doses of 0, 5, 15, or 30 mg/kg/day on gestation days (GD) 6-15, inclusive. On GD 19, dams were sacrificed and the fetuses removed. Approximately one-third of the fetuses from each litter were sectioned and examined for visceral malformations/variations. The remaining two-thirds of each litter were "examined externally" and processed and examined for skeletal malformations/variations.

Two high-dose animals died prematurely. Clinical signs of toxicity, including tremors, convulsions, urine stains, excitability, and anorexia, were reported for one high-dose animal. However, it was not possible to correlate clinical signs with death since individual animal data were not included. No other clinical signs of toxicity were reported. Body weight gains by the mid- and high-dose dams were 76% and 23%, respectively, of the control levels during the treatment interval with both groups attaining statistical significance ($p \leq 0.05$). Overall body weight gain by the high-dose group was 69% (p 0.05) of the controls. Food consumption by the high-dose group was 47% of the control level during GD 6-11. Body weight gains by the low-dose group and food consumption for the low- and mid-dose groups were similar to the controls throughout the study. Gross necropsy data, other than uterine data, for the dams were not provided.

Therefore, the maternal toxicity LOAEL is 15 mg/kg/day based on decreased body weight gain. The maternal toxicity NOAEL is 5 mg/kg/day.

No treatment-related effects were observed between the control group and the treated groups for number of corpora lutea, number of implantation sites, live fetuses/dam, pre- and post-implantation losses, fetal body weights, or fetal crown-rump lengths. No treatment-related visceral or skeletal malformations/variations were observed in any of the fetuses. Results of external examination were not reported.

Therefore, the developmental toxicity NOAEL is >30 mg/kg/day and the developmental toxicity LOAEL was not identified.

This study is classified as **Unacceptable/nonguideline** and does not satisfy the requirements for a developmental toxicity study (83-3a) in rats. Several deficiencies were noted in the conduct of this study: the subcutaneous route is not the preferred method of administration, percent purity of the test article was not given, dosing solutions were not analyzed for concentration, stability, or homogeneity, less than 20 litters/group were available for evaluation, and much of the individual maternal and fetal data were not included. However, this study may be used as supplemental information.

This study was classified unacceptable; however, a new developmental toxicity study in rabbits is not required and thought to not be beneficial for the following reasons:
1) The developmental toxicity study in rabbits and rats using a subcutaneous route of administration shows no developmental effects at the maternally toxic dose.
2) The skeletal effects observed in the developmental toxicity study in rats, with gavage as the route of administration, are within historical controls.
3) More severe maternal effects are seen in the rabbit study with subcutaneous administration.
4) The rat appears to be the more sensitive species for developmental effects.

5) A developmental neurotoxicity study has already been submitted.

Executive Summary: In a developmental toxicity study (MRID 42808002), 13 presumed pregnant New Zealand white rabbits per group were administered Lindane (purity not given; Batch No. 6801/403) by gavage in 0.5% carboxymethyl-cellulose at doses of 0, 5, 10, or 20 mg/kg/day on gestation days (GD) 6-18, inclusive. On GD 29, dams were sacrificed, subjected to gross necropsy, and all fetuses examined for visceral and skeletal malformations/variations. Data from external examination of the fetuses was not included.

All does survived to scheduled sacrifice. No treatment-related clinical signs of toxicity were observed. Maternal body weight and food consumption were similar between the treated and control groups. Gross necropsy was unremarkable. Organ weights were similar between the treated and control groups.

Therefore, the maternal toxicity NOAEL is >20 mg/kg/day and the maternal toxicity LOAEL was not identified.

No treatment-related effects were observed in any dose group for number of corpora lutea, number of implantation sites, live fetuses/dam, pre- and post-implantation losses, fetal body weights, or fetal sex ratios. No treatment-related visceral or skeletal malformations/variations were observed in any of the fetuses.

Therefore, the developmental toxicity NOAEL is >20 mg/kg/day and the developmental toxicity LOAEL was not identified.

This study is classified as **Unacceptable/not upgradable** and does not satisfy the requirements for a developmental toxicity study (83-3b) in rabbits. Maternal and developmental toxicity LOAELs were not identified and the highest dose did not approach the limit dose. Therefore, dose selection was considered inadequate. Doses were based on the results of a subcutaneous study in the rabbit (MRID 00062658) which is not a valid method for selecting doses for an oral study. Several other deficiencies were noted in the conduct of this study: percent purity of the test article was not given, dosing solutions were not analyzed for concentration, stability, or homogeneity, and much of the individual animal data were not included.

Executive Summary: In a developmental toxicity study (MRID 00062658), 15 presumed pregnant New Zealand white rabbits per group following artificial insemination were administered Lindane (purity and Batch No. not given) by subcutaneous injection in corn oil (0.5 ml/kg) at doses of 0, 5, 15, or 45 mg/kg/day on gestation days (GD) 6-18, inclusive. Due to excessive toxicity, the high dose was reduced to 30 mg/kg/day after GD 9. On GD 29, dams were sacrificed, subjected to gross necropsy, and all fetuses examined for visceral and

skeletal malformations/variations. Data from external examination of the fetuses was not included.

One mid-dose dam aborted and died on GD 21 and 14/15 high-dose animals died between GD 10 and 26. The high-dose group was then discontinued due to excessive mortality. Decreased activity and immobilized rear quarters were observed in the mid-dose group (frequency and number affected not reported). No clinical signs of toxicity were observed in the low-dose group. During GD 6-20, does in the mid-dose group had a body weight loss of 126.7 g as compared with a body weight gain of 218.0 g by the controls. Body weight loss was accompanied by "markedly lower" food consumption by the mid-dose animals. Body weight changes and food consumption for the low-dose group were similar to the controls throughout the study.

It appeared that does in the mid- and high-dose group had differences in the texture of the liver, however, data from gross necropsy were difficult to interpret due to poor copy quality of the original report.

Therefore, the maternal toxicity LOAEL is 15 mg/kg/day based on clinical signs of toxicity, death, and reduction in body weight. The maternal toxicity NOAEL is 5 mg/kg/day.

No treatment-related effects were observed between the control group and the treated groups for number of corpora lutea, number of implantation sites, live fetuses/dam, pre- and post-implantation losses, fetal body weights, or fetal crown-rump distances. No treatment-related visceral or skeletal malformations/variations were observed in any of the fetuses. Abortion by one mid-dose doe was assumed to be due to excessive maternal toxicity and not to a direct effect on the embryos or fetuses.

Therefore, the developmental toxicity NOAEL is >15 mg/kg/day and the developmental toxicity LOAEL was not identified.

This study is classified as **Unacceptable/not upgradable** and does not satisfy the requirements for a developmental toxicity study (83-3b) in rabbits. Several deficiencies were noted in the conduct of this study: the subcutaneous route is not the preferred method of administration, excessive toxicity occurred at the high-dose, percent purity of the test article wast not given, dosing solutions were not analyzed for concentration, stability, or homogeneity, and much of the individual maternal and fetal data were not included. However, these study results may be used in conjunction with the oral developmental toxicity study in rabbits (MRID 42808002) as supplemental information.

Study: Developmental Neurotoxicity Study

Executive Summary: See Short-Term Dermal (1-7 days); Section 2.3.2

5.4 Reproductive Toxicity

Executive Summary: In a multigeneration reproductive toxicity study (MRID 42246101), Lindane (99.5% a.i.; Batch No. DA433) was administered to groups of 30 male and 30 female Charles River CD rats at dietary concentrations of 0, 1, 20, or 150 ppm (0.087, 1.71, and 13.05 mg/kg/day, respectively) during the per mating period for two generations. One litter was produced in each generation. F_1 pups chosen as parental animals were weaned onto the same diet as their parents. Test or control diets were administered to the F_0 and F_1 parental animals for 71 and 70 days, respectively, before the animals were mated within the same dose group. All animals were continuously exposed to test material either in the diet or during lactation until sacrifice.

Premature sacrifices or intercurrent deaths of two F_0 animals and five F_1 animals were considered incidental to treatment; all other F_0 and F_1 males and females survived to terminal sacrifice. No treatment-related clinical signs of toxicity were observed in males or females of either generation at any time during the study. No treatment-related effects on body weights, body weight gains, food consumption, or food efficiency were observed for the F_0 and F_1 males and females during premating. Gross necropsy and histopathology of females was unremarkable.

During gestation days 10-13, mean body weight gain by the high-dose F_0 females was significantly reduced (11%). Mean body weight gains by the high-dose F_0 females were also significantly lower on lactation day 1 (interval not specified) as compared to the controls, but recovery was apparent by weaning. No treatment-related changes in body weights or body weight gains were observed in the F_1 females during gestation or lactation.

High-dose male rats of both generations had a significantly ($p \leq 0.01$) increased incidence of pale kidneys (10/29 F_0 males and 10/30 F_1 males) as compared with the controls (0/30 and 0/28, respectively). Areas of change on the kidneys (not defined) were observed in 7/29 high-dose F_0 males compared with 2/30 controls and in 4/30 mid-dose F_1 males and 5/30 high-dose F_1 males compared with 1/28 controls. Significantly ($p \leq 0.01$) increased incidence of hydronephrosis was observed in high dose F_1 males (7/30) as compared to controls (0/28). Absolute and relative kidney weights of the mid- and high-dose F_0 males and the high-dose F_1 males were significantly ($p \leq 0.01$) increased as compared with the controls.

F_0 and F_1 males in the mid- and high-dose groups had significantly ($p \leq 0.01$) increased incidences of chronic interstitial nephritis, cortical tubular cell regeneration, hyaline droplets in proximal tubules, tubular necrosis with exfoliation and cellular casts, and cortical tubular casts (n.s.). These changes are characteristic of alpha 2 globulin accumulation, which is specific to male rats.

Increased absolute and relative liver weights, accompanied by hepatocellular hypertrophy, in the mid- and high-dose males and females of both generations were considered adaptive and of no biological significance.

Therefore, the LOAEL for systemic toxicity is 150 ppm based on decreased body weight gains by the F_0 females during gestation. The systemic toxicity NOAEL is 20 ppm. In addition, the LOAEL for male rats is 20 ppm based on increased kidney weights and histopathological lesions in the kidney characteristic of alpha 2u globulin accumulation; the NOAEL for males is 1 ppm.

Mating, fertility, gestation survival (postimplantation index), and liveborn indices, mean precoital interval, and mean gestation length were similar between the treated and control groups of both generations. The sex distribution was not affected by the test material. Mean litter sizes of the treated groups were not different from the controls throughout lactation for both generations. Viability indices for the high-dose F_1 and F_2 pups were 81% and 85%, respectively, compared with 96% for the controls. This reduction in survival on lactation day 4 was due to the death or sacrifice (for humane reasons) of three F_1 litters and two F_2 litters. No treatment-related clinical signs of toxicity were observed in the pups of either generation during lactation. Pup necropsy was unremarkable.

Body weights of the low- and mid-dose F_1 and F_2 pups were similar to the controls throughout lactation. Body weights of the high-dose pups of both generations were significantly ($p \leq 0.01$) less than the controls on lactation days 1 and 25. In high-dose F_2 pups, the onset and completion of tooth eruption and completion of hair growth were significantly ($p \leq 0.01$) delayed 10.5%, 11.6%, and 24%, respectively, as compared with the controls.

Therefore, the LOAEL for reproductive toxicity is 150 ppm based on reduced pup body weights and decreased viability in both generations and delayed maturation of the F_2 pups. The reproductive toxicity NOAEL is 20 ppm.

This study is classified as **Acceptable/guideline** and satisfies the guideline requirements for a reproduction study (83-4) in rats. No major deficiencies were identified in the conduct of this study.

5.5 Additional Information from Literature Sources

Karmaus, W. et al, Reduced Birthweight and Length in the Offspring of Females Exposed to PCDFs, PCP, and Lindane. Environmental Health Perspectives; 103(12). 1995. 1120-1125.

The objective of this study was to investigate a broad range of adverse health outcomes and their

potential association to wood preservative used in daycare centers. This article focuses on reproductive effects. A sample of 221 exposed teachers was provided by the employer's liability insurers. A comparison group (n = 189) insured by the same two organizations was recruited from nonexposed daycare centers. In a face-to-face interview, job history and reproductive history of 398 female teachers were ascertained. Data on exposure were provided, including measurements on concentration of pentachlorophenol (PCP) and lindane in wood panels, and of PCP, lindane, polychlorinated dibenzo-p-dioxins and dibenzofurans in indoor air. An exposure matrix based on individual job history, independent exposure information from each center, and reproductive history was set up with regard to the vulnerable time windows for each pregnancy. Using this approach, 49 exposed and 507 nonexposed pregnancies were identified, including 32 exposed and 386 nonexposed live births. For subgroup analyses the observations were restricted to independent pregnancies, excluding multiple and consecutive births. The data were analyzed with linear regression techniques, taking confounders into account. The crude median difference between exposed and nonexposed was 175 g in birthweight and 2 cm in length. Controlling for confounders, the results show a significantly reduced but weight ($p = 0.04$) and length ($p = 0.02$) in exposed pregnancies, even after restricting the data to independent pregnancies and pregnancies for which data could be validated from the mother's health cards. These differences were not explained by differences in gestational age indicating that a toxic effect, which could cause small-for date newborns, might have affected the fetus.

Rivera, S. et al, Behavioral Changes Induced in Developing Rats by an Early Postnatal Exposure to Lindane. Neurotoxicity and Teratology, 12(6). 1990. 591-595

The purpose of this study was to determine whether the behavioral developmental pattern was altered by an early postnatal exposure to lindane. Male and female offspring of Wistar rats were daily orally administered with a nonconvulsant dose of lindane (10 mg/kg) during 7 days either the 1st or the 2nd postnatal week days. Effects on pups were evaluated with a reduced developmental neurotoxicological test battery. Body weight evolution, neuromotor reflexes (surface righting, cliff avoidance and tail hang reflex) and spontaneous motor activity were analyzed from day 1 after birth up to day 28. The body weight pattern was unaffected by treatment with lindane and no signs of overt toxicity were observed. Lindane-treated pups showed an increased positive response of the neuromotor reflexes. Furthermore, lindane produced hyperactivity, especially manifested between days 12 and 16. A peak of activity was reached at day 16 in lindane-treated group, while control animals had a maximum between days 20 and 24. These results suggest that low nonconvulsant doses of lindane may induce behavioral changes in developing rats.

Sircar, S. et al, Lindane (gamma-HCH) Causes Reproductive Failure and Fetotoxicity in Mice. Toxicology 59(2). 1989. 171-177.

Lindane (gamma-Hexachlorocyclohexane) was orally given to pregnant Swiss female mice at various stages of pregnancy. During early pregnancy (1-4 days of gestation), the insecticide caused

total absence of any implantation site, while given during mid pregnancy (6-12 days of gestation), lindane caused total resorption of fetuses. Lindane administration during late pregnancy (14-19 days of gestation) resulted in death of all pups either within 12 h (high-dosed group) or 5 days (low-dosed group) of parturition. Body weight of such pups were also highly reduced. When estrogen was given together with lindane at early pregnancy, implantation was normal, although subsequent fetal development was adversely affected. Progesterone, unlike estrogen, could not correct lindane-induced failure in implantation. On the other hand, when estrogen and progesterone were simultaneously given to lindane-fed mice during early pregnancy, both implantation and subsequent fetal development became comparable to normal mice. The insecticide besides being fetotoxic, thus appears to cause steroid hormone deficiency resulting in reproductive and developmental failure.

Pompa, G. et al, Transfer of Lindane and Pentachlorobenzene From Mother to Newborn Rabbits, Pharmacology and Toxicology; 74(1). 1994. 28-34.

After administration of gamma-hexachlorocyclohexane (lindane) (30 mg/kg) to sixteen pregnant rabbits, the transfer and distribution of this insecticide and its metabolite pentachlorobenzene, in foetuses and newborns at the 5th, 10th and 20th days after birth, were investigated. Over one lactation the mothers excreted via the milk about 30% of the lindane present in tissues at the 28th day of pregnancy. The total amount of lindane transferred via milk to 5 day-old newborns was higher than that transferred across the placenta during pregnancy. Lindane concentrations in newborns decreased in spite of the efficient transfer to off-spring by lactating mothers. This cannot be explained by growth alone and indicates that newborns are able to actively metabolize the insecticide. The pentachlorobenzene metabolite produced after lindane administration to the mothers crossed the placental barrier with difficulty during pregnancy, but was readily transferred to off-spring via milk. Pentachlorobenzene levels in neonates increased during lactation by transfer and also as a consequence of endogenous production. At the 20th day of lactation the pentachlorobenzene concentration in maternal and foetal tissues was higher than that of lindane.

5.6 Determination of Susceptibility

No quantitative or qualitative evidence of increased susceptibility of rat or rabbit fetuses to *in utero* exposure in developmental toxicity studies. In the two generation reproductive study, there was qualitative evidence of an increased susceptibility to exposure to lindane by pups. In the parental animals, toxicity was seen in the form of reduction in body weight gain during gestation while offspring toxicity was correlated with decreases in pup viability and pup body weight in the F_1 and F_2 generations as well as delayed maturation in the F_2 generation. Evidence for quantitative increase in susceptibility could not be ascertained due to the wide spread in the doses tested.

In the DNT study, there is supporting evidence of a qualitative and quantitative increase in susceptibility. At the high dose (13.7 mg/kg/day) , animals in the F_0 generation have a reduced

body weight and body weight gain while at the mid-dose (5.6 mg/kg/day) F_1 and F_2 animals have a reduced survival rate, decreased body weights and body weight gains during lactation, increased motor activity, and decreased motor activity habituation as compared to controls. The open literature also contains citations which suggest an increase in susceptibility of fetuses and young animals to exposure to lindane (see Section 5.5).

6 HAZARD CHARACTERIZATION

Lindane is a moderately toxic compound in EPA toxicity class II. It is neither an eye nor dermal sensitizer. Labels for products containing it must bear the Signal Word WARNING. Some formulations of lindane are classified as Restricted Use Pesticides (RUP), and as such may only be purchased and used by certified pesticide applicators. Lindane is no longer manufactured in the U.S., and most agricultural and dairy uses have been canceled by the EPA because of concerns about the compound's potential to cause cancer.

The primary effect of Lindane is on the nervous system; in both acute, subchronic, and developmental neurotoxicity studies and chronic toxicity/oncogenicity study, Lindane appears to cause neurotoxic effects including tremors, convulsions and hypersensitivity to touch. This is further corroborated by the published literature in which human exposure has been seen to produce neurologic effects. Lindane also causes renal and hepatic toxicity via the oral, dermal and inhalation routes of exposure as seen in subchronic, 2-generation reproduction and chronic toxicity studies in the rat.

In developmental toxicity studies, no developmental effects were seen at levels where maternal toxicity was evident. In the rat developmental study, the developmental effects (extra rib and total skeletal variations) were seen at dose levels (20 mg/kg/day) greater than maternal toxicity (10 mg/kg/day). In the reproductive toxicity study, both systemic and developmental LOAELs are 13 mg/kg; however a qualitative difference in maternal and offspring effects (reduced body weight of maternal animals and reduced viability and delayed maturation in pups) indicates an increased susceptibility to exposure. This is further corroborated by a developmental neurotoxicity study in which a qualitative and quantitative increase in susceptibility is seen. At the high dose (13.7 mg/kg/day) , animals in the F_0 generation have a reduced body weight and body weight gain while at the mid-dose (5.6 mg/kg/day) F_1 and F_2 animals have a reduced survival rate, decreased body weights and body weight gains during lactation, increased motor activity, and decreased motor activity habituation as compared to controls.

According to the TES committee report (1994, Doc 013460), Lindane has not been classified by the HED Cancer Peer Review Committee. It was determined by the RfD/Peer Review Committee (8/25/93) that: "The mouse carcinogenicity data were considered insufficient because of major deficiencies associated with all studies available." Lindane however had been previously classified by the Cancer Assessment Group of the Office of Research and Development (memorandum dated 7/23/85 from R.E. McGaughy to Anne Barton) as a group B2/C carcinogen based on increased incidence of mouse liver tumors. The upper-bound slope of the dose-response was given in that memorandum as $Q1^* = 1.1\ (mg/kg/day)^{-1}$. A new

mouse oncogenicity study is expected in December 2000.

Lindane does not appear to be mutagenic. The available mutagenicity studies are negative; they include a dominant lethal mutation assay, sister chromatid exchange assay and mammalian cell culture gene mutation in V79 cells. IPCS also states that Lindane does not appear to have mutagenic potential.

7 DATA GAPS

none

8 ACUTE TOXICITY

STUDY TYPE	MRID	CATEGORY	RESULT
81-1 Acute oral	00049330	II	LD_{50} 88 mg/kg - males 91 mg/kg - females
81-2 Acute dermal	00109141	II	LD_{50} 1000 mg/kg - males 900 mg/kg - females
81-3 Acute inhalation	Acc. 263946	III	LC_{50} 1.56 mg/L both sexes
81-4 Eye irritation	Acc. 263946	III	PIS = 0.6 no corneal involvement irritation cleared after 24 hours
81-5 Dermal irritation	Acc. 263946	IV	PIS = 0 not an irritant
81-6 Dermal sensitization	Acc. 263946	NA	not a sensitizer

9 TOXICOLOGIC PROFILE

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY TYPE/ MRID
Acute Dietary- general population	NOAEL= 6 mg/kg UF = 100	LOAEL is 20 mg/kg based on increased grip strength, increased Motor Activity	Acute Neurotoxicity in Rats/ 44769201
Acute Dietary-females 13-50	NOAEL= N/A UF = N/A	No relevant single exposure endpoint was identified.	N/A
Acute RfD (Gen. Pop.) = 0.06 mg/kg/day Acute RfD (Females 13-50) = N/A			
Chronic Dietary	NOAEL=10 ppm (0.47 mg/kg/day) UF = 100	LOAEL is 100 ppm (4.81 mg/kg/day) periacinar hepatocyte hypertrophy, increased liver/spleen weigt, increased platelets	Chronic Feeding and Carcinogenicity in Rats 41094101 41853701 42891201
	Chronic RfD = 0.047 mg/kg/day		
Cancer Risk [3]	**Q_1* = 1.1 (mg/kg/day)$^{-1}$**		
Short-Term [1] (Dermal)	NOAEL= 10 ppm (1.2 mg/kg/day)	LOAEL is 50 ppm based on reduced pup survival, decreased body weights and body weight gains during lactation, increased motor activity, and decreased motor activity habituation.	Developmental Neurotoxicity Study in Rats 45073501
Intermediate-Term [1] (Dermal)	NOAEL= 10 ppm (1.2 mg/kg/day)	LOAEL is 50 ppm based on reduced pup survival, decreased body weights and body weight gains during lactation, increased motor activity, and decreased motor activity habituation.	Developmental Neurotoxicity Study in Rats 45073501
Long-Term [1] (Dermal)	NOAEL=10 ppm (0.47 mg/kg/day)	LOAEL is 100 ppm (4.81 mg/kg/day) periacinar hepatocyte hypertrophy, increased liver/spleen weigt, increased platelets	Chronic Feeding and Carcinogenicity in Rats 41094101 41853701 42891201
Dermal Absorption Factor = 10%			
Short Term [1] (Inhalation)	0.5 mg/m^3 (0.13 mg/kg/day)	based on clinical signs (diarrhea, piloerection) seen at day 14 and continuing for 20 days	90-Day Inhalation Toxicity 00255003
Intermediate Term [1] (Inhalation)	0.5 mg/m^3 (0.13 mg/kg/day)	increased kidney weights in females and bone marrow effects (incr. reticul, incr myelo, decr. lympho.)	90-Day Inhalation Toxicity 00255003

Long Term [2] (Inhalation)	N/A	N/A	N/A

[1] An MOE of 100 was selected

[2] Exposure thru this route for this duration is not expected

[3] The Cancer Risk will be re-evaluated upon review of the Mouse Carcinogenicity Study submitted in December 2000